JOURNAL OF VIROLOGY, June 1995, p. 3704–3711 Vol. 69, No. 6
0022-538X/95/$04.00+0

Enhancement of Feline Immunodeficiency Virus Infection after Immunization with Envelope Glycoprotein Subunit Vaccines

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Received 4 January 1995/Accepted 9 March 1995

Cats were immunized three times with different recombinant feline immunodeficiency virus (FIV) candidate vaccines. Recombinant vaccinia virus (rVV)-expressed envelope glycoprotein with (vGR657) or without (vGR657x15) the cleavage site and an FIV envelope bacterial fusion protein (β-Galactosidase–Env) were incorporated into immune-stimulating complexes or adjuvanted with Quil A. Although all immunized cats developed antibodies against the envelope protein, only the cats vaccinated with the rVV-expressed envelope glycoproteins developed antibodies which neutralized FIV infection of Crandell feline kidney cells. These antibodies failed to neutralize infection of thymocytes with a molecularly cloned homologous FIV. After the third immunization the cats were challenged with homologous FIV. Two weeks after challenge the cell-associated viral load proved to be significantly higher in the cats immunized with vGR657 and vGR657x15 than in the other cats. The cats immunized with vGR657 and vGR657x15 also developed antibodies against the Gag proteins more rapidly than the cats immunized with β-Galactosidase–Env or the control cats. This suggested that immunization with rVV-expressed glycoprotein of FIV results in enhanced infectivity of FIV. It was shown that the observed enhancement could be transferred to naive cats with plasma collected at the day of challenge.

Feline immunodeficiency virus (FIV) is a T-lymphotropic lentivirus that causes feline AIDS, which is similar to AIDS in humans (1, 3, 9, 12, 20, 29, 39, 41, 45, 51). The similarities between FIV and human immunodeficiency virus (HIV) on the one hand and between the pathogeneses of the syndromes they cause on the other hand have led to the use of FIV infection of cats as an animal model to evaluate the potential of preventive and therapeutic measures for HIV infection in humans. Since FIV infection is widespread among cats all over the world, the development of preventive and therapeutic measures for feline AIDS is also of major veterinary importance.

Different vaccination strategies for lentivirus infections have been evaluated with varying degrees of success. Most attempts to develop candidate vaccines against lentivirus infections were without success (for a review, see reference 22). Nevertheless, in some experiments chimpanzees were successfully vaccinated against HIV type 1 (HIV-1) (4) and macaques were successfully vaccinated against HIV-2 or simian immunodeficiency virus (SIV) infections with candidate whole inactivated virus, live attenuated virus, recombinant virus, subunit virus vaccines, or combinations of these candidate vaccines (6, 7, 17, 21, 28, 30, 32, 38). The mechanisms by which protective immunity was established in these systems are not well understood at present, although there are indications for a major role of both virus-neutralizing (VN) antibodies and cell-mediated immunity (10, 24, 31). However, contradictory data have also been obtained in some of these studies (2, 5, 16, 18, 36). In the macaque SIV model it was shown that at least part of the protective immunity induced could be attributed not to virus-specific but rather to cell-specific antibodies (23, 46). In the SIV system it was recently shown that vaccine-induced protection against infection with SIV-infected cells correlated with the presence of a certain major histocompatibility complex class I genotype of the monkeys, indicating the involvement of major histocompatibility complex class I-restricted cytotoxic T-lymphocyte responses (11).

Several approaches to develop a preventive vaccine against FIV infection have also proven to be unsuccessful (16, 48). However, Yamamoto et al. reported the induction of protective immunity in cats against homologous and to a lesser extent also to heterologous FIV challenge, by vaccination with inactivated whole virus or FIV-infected cells (49, 50). This protective effect could be transferred to naive cats with plasma from vaccinated animals, indicating that antibodies may be at the basis of this protective immunity (13). It was shown that serum antibodies against FIV envelope glycoproteins, with different VN domains, correlated more with protective immunity than did antibodies to other viral proteins.

FIV vaccines based on recombinant envelope proteins would have clear advantages over inactivated or attenuated virus vaccines. However, so far vaccination strategies using FIV purified envelope glycoproteins or fractions of these proteins as immunogens have failed (16, 26). Here we report the results of a series of vaccination experiments in cats with different envelope proteins of FIV, expressed by recombinant vaccinia viruses (rVV) or as a bacterial fusion protein and presented in the context of different adjuvant systems. The most striking finding of these studies is that vaccines containing intact envelope glycoprotein induced enhancement of infectivity rather than protective immunity against homologous FIV infection. The observed enhancement could be transferred to naive cats with the plasma of the vaccinated cats.

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MATERIALS AND METHODS

Cells and challenge virus. Peripheral blood mononuclear cells (PBMC) and thymocytes were derived from an 8-week-old specific-pathogen-free (SPF) cat (42). These cells were stimulated with concanavalin A (5 μg/ml) in culture medium (RPMI 1640 [GIBCO, Gaithersburg, Md.], penicillin [100 IU/ml], streptomycin [100 μg/ml], L-glutamine [2 mM], 2-mercaptoethanol [2×10^{-5} M], interleukin-2 [100 IU/ml]) and 10% fetal calf serum. After 3 days the cells were washed and cultured further in culture medium. An FIV-susceptible clone of Crandell feline kidney (CrFK) cells, named CrFK 1D10, was kindly provided by N. Pedersen (51). The FIV AM19 strain was isolated from PBMC of a cat naturally infected with FIV (42). Concanavalin A- and interleukin-2-stimulated cells were infected with FIV AM19. When FIV antigen was detected in culture supernatant by enzyme-linked immunosorbent assay (ELISA) (40), it was filtered through a 220-nm-pore-size filter, aliquoted, and stored at −135°C. This FIV stock was titrated in vivo. Groups of four SPF cats were inoculated intramuscularly with 0.5 ml of 1:100, 1:400, 1:1,600, 1:6,400, or 1:25,600 dilutions of the FIV stock. All cats receiving 1:100 and 1:400 dilutions became infected by 4 to 8 weeks postinfection as shown by seroconversion and virus isolation. Three of the four cats inoculated with 1:1,600-diluted FIV stock and one of the four cats inoculated with 1:6,400-diluted stock became virus isolation positive and seropositive. One 50% cat infectious dose (CID_{50}) was therefore estimated to be 0.5 ml of a 1:3,200 dilution of the FIV stock.

FIV AM6c was isolated from PBMC of a cat naturally infected with FIV and adapted to replicate in CrFK 1D10 cells as previously described (43). *env* gene sequence homology of FIV AM6c and FIV19k1 is 94.8%. CrFK 1D10 cells were infected with FIV AM6c, and after 6 days the culture medium was refreshed. After another 4 days of culture, when FIV antigen was detected in the culture supernatant, it was filtered through a 220-nm-pore-size filter and stored in aliquots at −135°C. This FIV stock was titrated in CrFK 1D10 cells and the highest dilution (20 $TCID_{50}$s) which consistently resulted in detectable antigen production within 8 days was used in the VN assay (see below).

Preparation of candidate FIV vaccines. The envelope glycoproteins of FIV AM19 were expressed by an rVV in BHK cells either in their native form (vGR657) or after deletion of the cleavage site between the surface (SU) and the transmembrane (TM) proteins (vGR657x15) to facilitate incorporation into immune-stimulating complexes (iscoms) (33). After lectin purification these proteins were incorporated into iscoms, which resulted in two iscom preparations—vGR657 and vGR657x15 iscoms—as previously described (33). An 1,870-bp fragment of the envelope gene was excised from pBluescript, containing the whole envelope gene, by using the restriction enzymes *Bam*HI and *Bgl*II (nucleotide positions 350 and 2220, respectively) and subcloned into *Bam*HI-digested pEX vector (Stratagene, La Jolla, Calif.). This vector allows inducible expression of proteins as β-galactosidase (β-Gal) fusion protein. This protein was partially purified as inclusion bodies, solubilized, and mixed with Quil A as an adjuvant.

SIV envelope glycoprotein iscoms were prepared by E. Hulskotte using a method similar to that used for the cleavage site-deleted FIV envelope glycoprotein iscoms (19).

Vaccination and challenge infection of cats. Six groups of six SPF cats each were vaccinated three times subcutaneously according to the following schedule: group 1, vGR657 iscoms; group 2, vGR657x15 iscoms; group 3, vGR657x15 plus Quil A; group 4, β-Gal–FIV Env plus Quil A; group 5, SIV Env iscoms; and group 6, phosphate-buffered saline. The cats were vaccinated with 10 μg of protein at weeks 0, 4, and 10. Two weeks later the cats were challenged by the intramuscular route with 20 CID_{50}s of FIV AM19. PBMC and plasma samples were collected every 2 weeks postchallenge (p.c.) during an 8-week period.

Serological assays. Antibodies against the Gag proteins p24 and p17 were detected with a commercially available test kit, using recombinant p24 and p17 proteins (catalog number F1002-AB01; European Veterinary Laboratory B.V., Woerden, The Netherlands). Antibody titers were expressed as the optical density at 450 nm (OD_{450}) value of the serum multiplied by the dilution divided by three times the OD_{450} value of the negative control serum.

Antibodies against the envelope protein were detected by ELISA using synthetic peptides and bacterial fusion proteins, representing different regions of the envelope protein as shown in Fig. 1. The synthetic peptides were purchased from European Veterinary Laboratory B.V. (catalog numbers: SU peptide, EVS-000-PE-003; TM peptide, EVS-000-PE-004). The first synthetic peptide contains the immunodominant VN epitope within variable region 3 spanning amino acid residues 396 to 412 of the surface protein of the Petaluma strain (SU peptide) (25). The second peptide contains a B-cell epitope between amino acid positions 695 and 706 of the transmembrane protein of the same FIV strain (TM peptide). Antibody titers were calculated as described for the Gag ELISA.

An ELISA based on the detection of antibodies to a series of bacterial fusion proteins as shown in Fig. 1 was performed as described elsewhere (8).

VN assays. VN serum antibodies were determined in two different VN assays. The feline lymphocyte VN assay was based on inhibition of infection of thymocytes with molecularly cloned FIV 19k1 as previously described (44). The CrFK VN assay was based on the inhibition of infection of CrFK 1D10 cells with FIV AM6c, which is adapted to replicate in these cells. For this assay CrFK 1D10 cells (3.5×10^3) were seeded into a 96-well plate in 100 μl of Dulbecco modified Eagle medium supplemented with 5% fetal calf serum. The next day the cells were washed and incubated for 1 h at 37°C with Polybrene (8 μg/ml) in Dulbecco modified Eagle medium supplemented with 5% fetal calf serum. A mixture of equal volumes of the diluted (see above) virus stock of FIV AM6c and twofold serial dilutions of heat-inactivated serum was incubated for 1 h at 37°C. Then the CrFK cells were washed and incubated with the virus-serum mixture at 37°C. After 24 h the CrFK cells were washed twice and propagated in Dulbecco modified Eagle medium supplemented with 2% fetal calf serum. After 8 days the culture supernatant was tested for the presence of FIV antigen by ELISA (40). The neutralizing antibody titer was expressed as the highest dilution of the serum still preventing FIV antigen production in the culture supernatant.

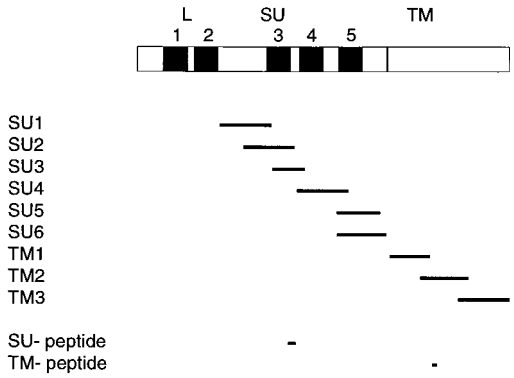


FIG. 1. Surface (SU1 to SU6) and transmembrane (TM1 to TM3) bacterial fusion products and SU and TM peptides used in the ELISA to detect antibodies against the envelope protein. The top bar represents the envelope protein of FIV, including the leader (L), surface (SU), and transmembrane (TM) proteins. The black boxes represent hypervariable regions in the envelope protein. The different bacterial fusion products and the peptides are indicated as bars.

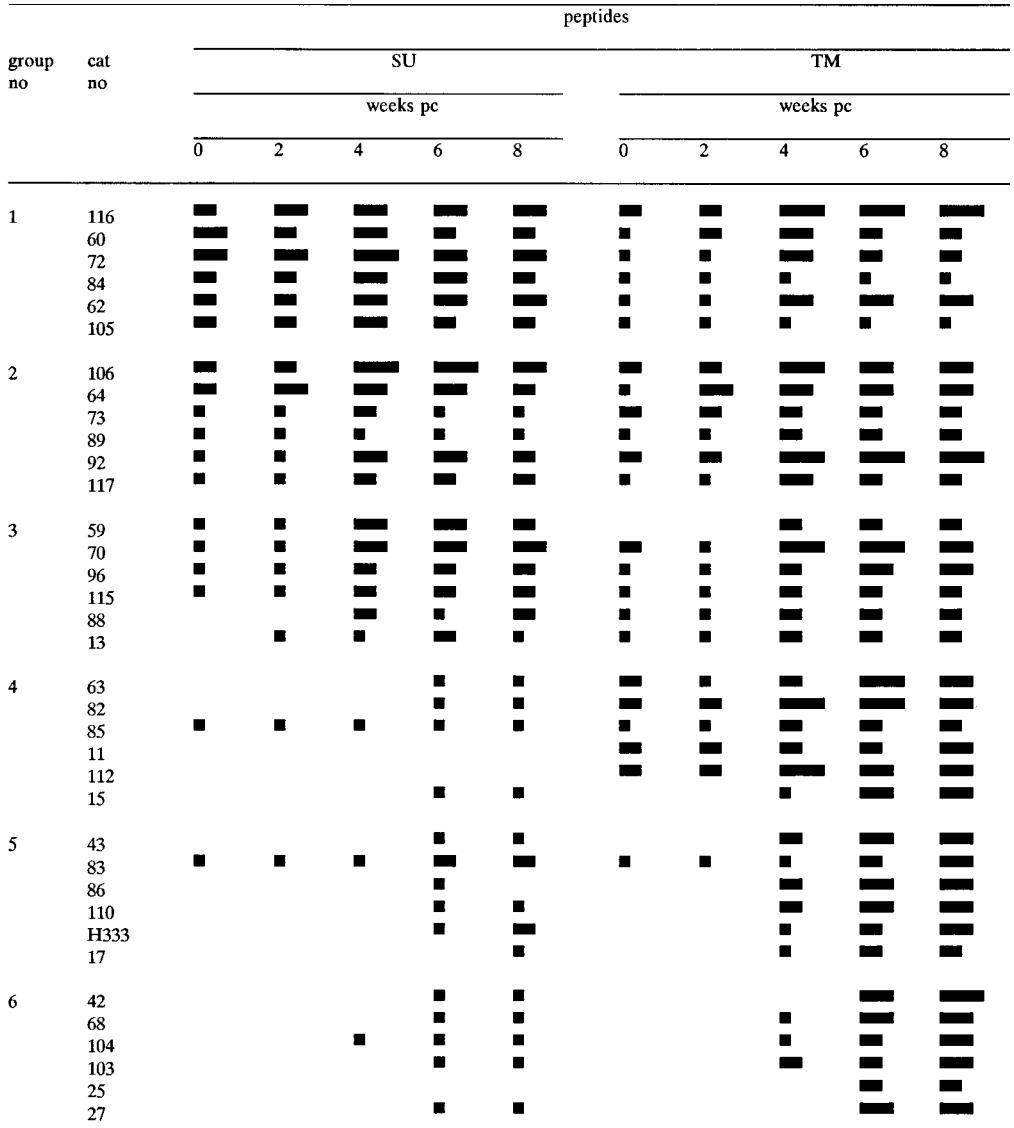
Cell-associated virus load: infectious center test. Serially diluted PBMC samples (1×10^3, 3×10^3, and 1×10^4 cells) were prepared from cats before and after challenge as previously described (42). These cells were cocultivated with 10^5 concanavalin A- and interleukin-2-stimulated PBMC from an SPF cat in eight duplicate wells. After 3 weeks the culture supernatants were tested for the presence of FIV antigen by ELISA. The number of infected cells in the PBMC in vivo was calculated from the in vitro culture by assuming that one infected cell gave rise to antigen production after cocultivation with concanavalin A- and interleukin-2-stimulated PBMC from an SPF cat, when one or more cultures tested in eight duplicate wells were negative for FIV antigen production.

Plasma transfer. A plasma pool was prepared by mixing equal volumes of the plasma samples derived from all the vaccinated cats from groups 1 and 2 at the day of challenge (plasma pool A). A second pool (plasma pool B) was prepared similarly by mixing plasma samples from all the cats of group 6 at the day of challenge. Two groups of four SPF cats, 10 weeks old, weighing between 800 and 1,250 g were used in the transfer experiment. Cats of group A and group B were injected intravenously with 7 ml of plasma pool A and plasma pool B, respectively. Six hours later the cats were challenged by the intramuscular route with 20 CID_{50}s of FIV AM19 as described above. PBMC and plasma samples of the cats were collected at weekly intervals and tested for viremia by virus isolation and for the development of plasma antibodies against the Gag protein.

RESULTS

Development of FIV-specific plasma antibodies upon vaccination. All plasma samples collected at the day of challenge were tested in the SU and TM peptide ELISA (Table 1, week p.c. 0). All the cats vaccinated with the vGR657 and vGR657x15 iscoms (groups 1 and 2) had developed plasma antibody titers to these peptides, ranging from 200 to 25,000, whereas all the cats vaccinated with Quil A-adjuvanted vGR657x15 (group 3) had developed antibody titers to at least one of the two peptides ranging from 50 to 3,000. Five of six cats vaccinated with the Quil A-adjuvanted bacterial FIV envelope fusion protein (group 4) had also developed serum antibody titers to the TM peptide, ranging from 50 to 3,000, whereas only one animal had developed a titer of 300 to the SU peptide. One cat of the two control groups, groups 5 and 6, showed an apparently nonspecific plasma antibody titer to the SU and TM peptides, which already existed before the start of the immunization procedure (not shown). Subsequently the

TABLE 1. Antibody response against SU and TM peptides in vaccinated cats at different times p.c.[a]



[a] SU peptide ELISA results: no symbol, titer < 200; ■, titer between 200 and 1,000; ■, titer between 1,000 and 5,000; ■, titer between 5,000 and 25,000; ■, titer > 25,000. TM peptide ELISA results: no symbol, titer < 50; ■, titer between 50 and 300; ■, titer between 300 and 3,000; ■, titer between 3,000 and 10,000; ■, titer > 10,000.

same plasma samples were tested in ELISA for antibody titers against the respective bacterial FIV envelope SU and TM fusion proteins (Table 2). Vaccination with the vGR657- and vGR657x15 iscoms (groups 1 and 2) resulted in high plasma antibody responses to all the SU and TM regions tested with the exception of the SU5 and TM1 regions, against which lower or no antibody levels were detected. Antibody levels induced with the native glycoprotein iscoms (group 1) and the cleavage site-deleted envelope iscoms (group 2) are comparable. The overall OD_{450} values induced with the cleavage site-deleted FIV envelope glycoprotein adjuvanted with Quil A (group 3) were lower than those found in the cats of groups 1 and 2. Vaccination with the β-Gal–FIV envelope fusion protein adjuvanted with Quil A failed to induce antibodies against the SU1, SU2, SU4, and SU5 regions. No significant levels of antibody to these fusion proteins were found in the cats of the two control groups (groups 5 and 6).

All the plasma samples collected at the day of challenge were also tested in the two VN antibody assays. None of the samples exhibited VN activity in the feline lymphocyte VN assay (not shown). However, VN antibodies could be demonstrated in cats of groups 1, 2, and 3 in the CrFK VN assay (Fig. 2). The VN titers in cats vaccinated with vGR657 iscoms (group 1) ranged from 160 to 640, with a mean titer of 506. Those of vGR657x15 iscom-vaccinated cats (group 2) ranged from 40 to 640 with a mean of 206, which is not significantly different from the values found for group 1. VN plasma antibody titers of cats vaccinated with the Quil A-adjuvanted vGR657x15 protein varied from 10 to 40, with a mean titer of 28, a value which is significantly lower than that found for

TABLE 2. Levels of antibody against envelope fragments in vaccinated cats at the day of challenge[a]

group no	cat no	Envelope regions								
		SU1	SU2	SU3	SU4	SU5	SU6	TM1	TM2	TM3
1	116	■	■	■	■		■		■	■
	60	■	■	■	■	■	■		■	■
	72	■	■	■	■	▪	■		■	■
	84	■	■	▪	■	▪	■		▪	■
	62	■	▪	■	■	▪	■		■	▪
	105	■	■	■	■	■	■		■	■
2	106	■	■	■	■	▪	■	▪	■	■
	64	■	■	■	■	▪	■		■	■
	73	■	■	■	■	▪	■	■	■	■
	89	■	■	▪	■		■		▪	■
	92	■	■	▪	■	▪	■	▪	■	■
	117	■	■	▪	■		■		■	■
3	59	■	■	▪	■		■	▪	■	
	70	■	■	▪	■		■	▪	■	
	96				■		■	■	■	▪
	115				■		■		■	
	88				■		■	▪	■	■
	13				▪				▪	
4	63			■			■	▪	▪	▪
	82			■			▪	■	■	▪
	85			■				▪		▪
	11			■			■	■	■	▪
	112			▪			▪	■	■	▪
	15			■			▪	■	▪	
5	43									
	83									
	86									
	110									
	H333									
	17									
6	42									
	68									
	104									
	103									
	25									
	27									

[a] No symbol, $OD_{450} < 0.4$; ■, OD_{450} between 0.4 and 0.8; ■, OD_{450} between 0.8 and 1.2; ■, $OD_{450} > 1.2$.

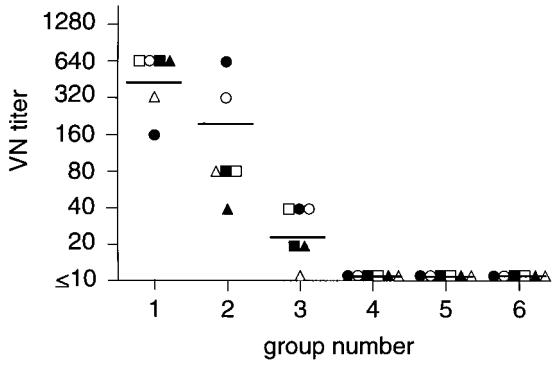


FIG. 2. Plasma VN antibody titers of the individual cats of the six different vaccination groups as measured in the CrFK VN assay at the day of challenge. The titers in the individual cats are indicated with different symbols. The mean titer per group is indicated by a bar.

groups 1 and 2 (Student t test; $P < 0.05$). In cats vaccinated with the β-Gal–FIV envelope fusion protein (group 4) and the cats of the control groups 5 and 6, no VN antibody response could be demonstrated at the day of challenge.

As expected, none of the cats had developed plasma antibodies to the FIV Gag protein at the day of challenge (Fig. 3).

Kinetics of FIV-specific plasma antibodies after FIV challenge. Plasma antibody titers against the SU and TM peptides of most of the cats immunized with the recombinant FIV envelope proteins (groups 1 to 4) increased 3- to 10-fold within 4 weeks after challenge infection. Within 8 weeks all the cats, including the control animals in groups 5 and 6, had developed anti-SU and anti-TM peptide plasma antibodies (Table 1). At 8 weeks p.c. the SU peptide-specific antibody titers in the cats vaccinated with rVV-expressed FIV envelope glycoprotein were significantly higher than those of the nonvaccinated cats (Table 1; $P < 0.05$).

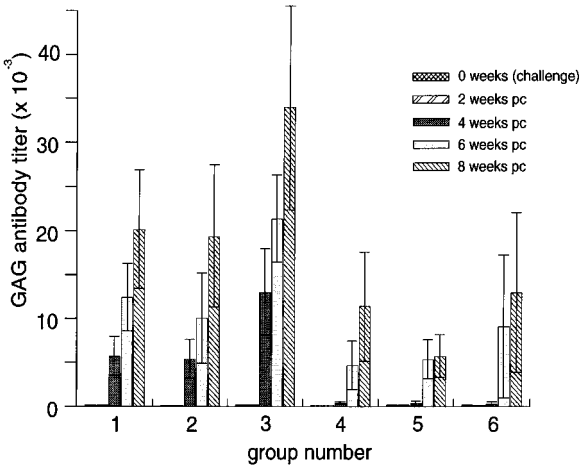


FIG. 3. Kinetics of the plasma antibody titer development against the Gag protein, in cats from the different vaccination groups. Mean anti-Gag titers at different times after challenge are presented.

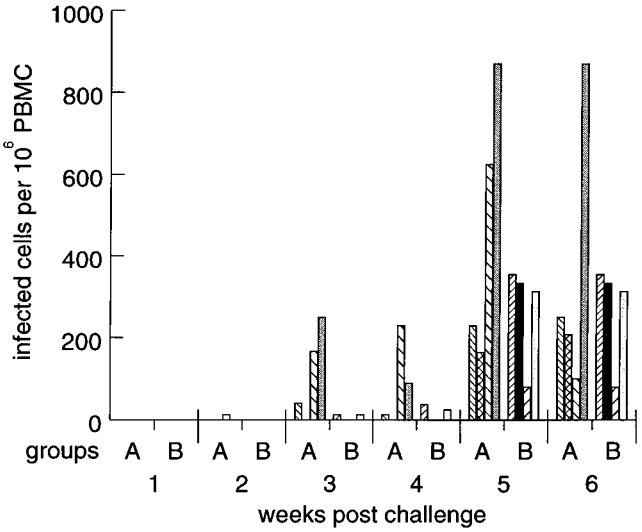


FIG. 5. Kinetics of numbers of FIV-infected cells per 10^6 PBMC in individual cats (indicated by different bars) transferred with plasma pool A (groups 1 and 2) or plasma pool B (group 6), measured over 6 weeks p.c.

Plasma antibodies to the FIV Gag protein could be detected 4 weeks after challenge in all the cats vaccinated with rVV-expressed FIV envelope glycoproteins (groups 1, 2, and 3) (Fig. 3). In the cats vaccinated with the β-Gal–FIV envelope fusion protein (group 4) and in the cats of the two control groups, groups 5 and 6, it took about 2 weeks longer before FIV Gag-specific antibodies were detected. Not only was the induction of FIV Gag-specific antibodies in cats of groups 1 to 3 faster, but also the 8-week p.c. plasma titers tended to be higher in these animals (Fig. 3).

Cell-associated virus load. Two weeks after infection FIV-infected PBMC were demonstrated in all the cats of groups 1 and 2 and in four of six cats of group 3 (Fig. 4). The numbers of FIV-infected PBMC varied from 50 to 210, 30 to 1,000, and 0 to 106 per 10^6 PBMC, respectively. In the other three groups no infected PBMC were demonstrated at this time. Two weeks later, FIV-infected PBMC could be demonstrated in all the cats of all groups. Although a lower average FIV load was observed in cats of group 4 compared with that in the other groups, the differences observed between the groups proved not to be significant.

Plasma transfer experiment. Pools of plasma collected at the day of challenge from the cats of groups 1 and 2 (pool A) and from group 6 (pool B) were prepared, and VN titers were determined. The titers, measured in the CrFK VN assay, were 320 and <10, respectively. Two groups of four SPF kittens were inoculated intravenously with 7 ml of plasma pool A or plasma pool B. No plasma VN antibodies could be detected 6 h after the transfer. FIV could be demonstrated in PBMC of one of four cats of group A (20 infected cells per 10^6 PBMC) but not in PBMC of cats of group B 2 weeks after challenge with 20 CID_{50}s of FIV AM19 (Fig. 5). Three weeks after challenge three of four cats of group A and two of four cats of group B exhibited cell-associated viremia, with significantly higher numbers of FIV-infected cells in the cats of group A ($P < 0.05$). The mean numbers of infected PBMC in the cats from groups A and B at 3 weeks p.c. were 115 and 6 per 10^6 PBMC, respectively (Fig. 5). At 4 weeks p.c. the mean numbers of FIV-infected cells were 111 and 16 per 10^6 PBMC, respectively. Five and six weeks after challenge FIV could be reisolated from PBMC from all cats of both groups with no clear differences in cell-associated virus load.

Four weeks after challenge, plasma antibodies to the Gag

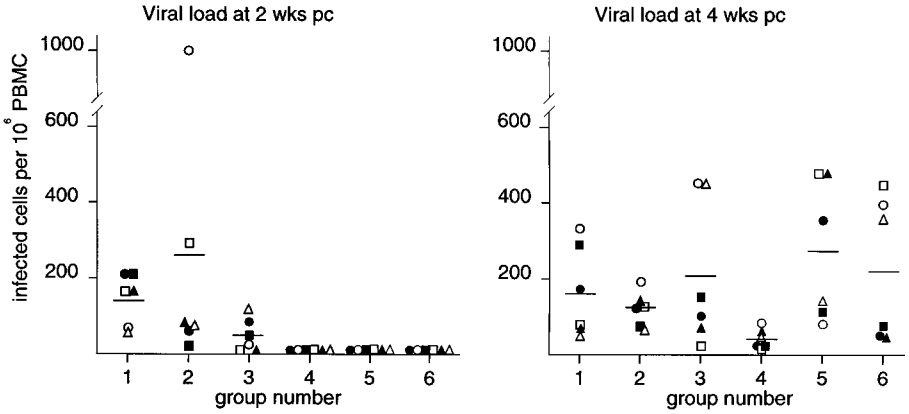


FIG. 4. Numbers of FIV-infected cells per 10^6 PBMC in the individual cats (indicated by different symbols) of the different vaccination groups at 2 and 4 weeks p.c. The mean numbers of infected PBMC per group are indicated by a bar.

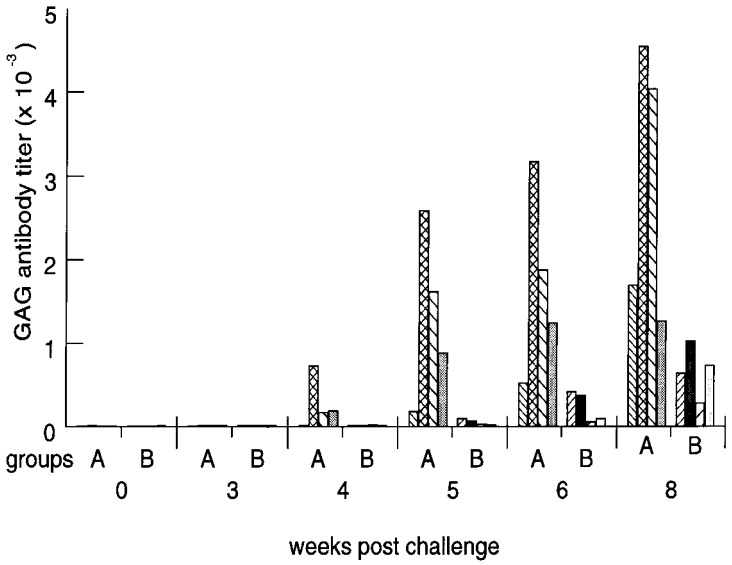


FIG. 6. Kinetics of plasma antibody titer development against the Gag protein in individual cats (indicated by different bars) transferred with plasma of pool A (groups 1 and 2) or plasma of pool B (group 6).

protein could be detected in three of four cats of group A (with ELISA titers ranging from 170 to 800) and in none of the cats of group B (Fig. 6). At 5 weeks p.c. anti-FIV Gag plasma antibodies were demonstrated in all cats of group A (titers from 180 to 2,600) and in only two of four cats from group B (titers from 70 to 100). In the plasma samples collected from all the cats of both groups at 6 and 8 weeks p.c., antibodies against the Gag protein were demonstrated. The titers were significantly higher ($P < 0.05$) in the cats of group A than those in the cats of group B from 4 weeks p.c. onward.

DISCUSSION

The present paper describes the evaluation of the potential of different candidate FIV vaccines in a vaccination challenge experiment in SPF cats. The most striking finding was that immunization with rVV-expressed FIV glycoproteins, resulting in VN plasma antibodies, led to enhanced FIV infection upon challenge in these cats (groups 1, 2, and 3). This was demonstrated by the more rapid development of PBMC-associated viremia and Gag-specific plasma antibodies. The enhancement effect could be transferred to naive cats with plasma collected from cats immunized with these candidate vaccines. In contrast, cats immunized with an FIV envelope bacterial fusion protein (group 4) did not develop VN plasma antibodies and developed PBMC-associated viremia and Gag-specific plasma antibodies with the same kinetics as the two control groups.

Comparison of the antibody levels induced in the first three groups of cats at the day of challenge indicated that the immunogenicity of vGR657 and vGR657x15 incorporated into iscoms was higher than that of vGR657x15 presented with Quil A. Both VN plasma antibodies and plasma antibodies to the different SU regions proved to be higher in the first two groups (Fig. 2; Table 2). Plasma antibody levels against most of the SU regions were lower or absent in group 4 at the day of challenge (Table 2). It is not clear why the presence of VN plasma antibodies at the day of challenge correlated not with protective immunity but rather with enhanced susceptibility to FIV infection. It should be noted, however, that the VN activity was demonstrable only in the CrFK VN assay and not in the feline lymphocyte assay. The latter assay should probably be considered more relevant in terms of protective immunity against in vivo FIV infection since, in contrast to feline kidney cells, feline lymphocytes are natural targets for FIV infection. In the transfer experiment it was shown that the enhancement phenomenon could be transferred to naive cats with the plasma of cats of groups 1 and 2. Although not formally proven, this indicated that the enhancement was mediated by FIV envelope-specific antibodies. The mechanism of enhancement proved to be operational at relatively high dilutions: after plasma transfer, no VN antibody activity could be demonstrated in the plasma of the kittens which subsequently showed enhanced FIV infectivity. It has also been shown for HIV-1 that antibody-dependent enhancement (ADE) could still be demonstrated at high dilutions (up to 1:65,000), whereas VN antibody activity can rarely be demonstrated at dilutions higher than 1:1,000 (35). As no reliable systems are available at present to quantify or even detect FIV-enhancing antibodies in vitro, it should be realized that all FIV-neutralizing antibodies found, per definition, should be considered to be the net result of neutralization and enhancement of FIV infectivity measured in vitro.

To date different mechanisms of ADE have been described for lentivirus infections. Complement- and Fc receptor-mediated ADEs have been shown to play a role in HIV-1, HIV-2, and SIV infections (14, 15, 27, 34, 47). Recently, another mechanism of ADE was described, in which antibodies neutralized or enhanced HIV-1, dependent on the phenotype of the virus involved (37). From our data it cannot be concluded which mechanism was involved in the observed enhancement of FIV infectivity. Indications for enhanced infectivity after FIV vaccination have been observed before (16). In the experiments of Yamamoto et al. (50) the presence of VN antibodies, demonstrated in an FeT1 cell (feline lymphoid cell line) VN assay, correlated with protective immunity rather than with enhancement of infectivity. This immunity could be transferred to naive animals with plasma of immunized animals (13). The main difference between their vaccination approach and ours is that in our experiments recombinant envelope proteins were used whereas Yamamoto et al. used inactivated whole virus or virus-

infected cells as immunogens. Since transfer experiments showed that probably plasma antibodies are involved in the observed mechanisms of enhancement and protection in both series of experiments, it may be speculated that the differences in configuration in which the FIV envelope glycoproteins were presented in both vaccines led to VN antibodies with different affinities. This may have had direct consequences for their in vivo effects.

Furthermore, the cell substrates used for the production of challenge viruses may have contributed to the observed differences in outcome of the vaccination experiments. The challenge virus used in our experiments was propagated in primary feline lymphocytes, whereas the challenge viruses in the experiments of Yamamoto et al. were propagated in a feline T-cell line (50). Like in the HIV-1 system, T-cell line-adapted FIV may be neutralized more efficiently than virus isolates from primary lymphocyte cultures, which may result in neutralization of the virus in the presence of enhancing antibodies. Furthermore, it may be speculated that, as was recently demonstrated for HIV-1 isolates from one individual (37), T-cell line-adapted FIV is less susceptible to ADE.

Taken together, it should be stressed that the mechanisms leading to the observed phenomenon of FIV enhancement upon vaccination and passive transfer are not fully understood at present. For the development of an effective FIV vaccine, the elucidation of the underlying mechanisms may be crucial. This may also lead to a more rational strategy for the development of HIV-1 vaccines.

ACKNOWLEDGMENTS

We thank Geert van Amerongen and Nico Schmidt for biotechnical assistance and Ellen Hulskotte for the SIV envelope iscoms. We also thank Rob van Herwijnen from the European Veterinary Laboratory for the SU and TM peptide ELISA, and we thank Conny Kruyssen for help in preparing the manuscript.

This work was supported by grants from Mallinckrodt Veterinary Ltd., Harefield, United Kingdom, and the Dutch Advisory Council on Health Research (RGO grant 88-90/89028).

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